region such that the overhangs in each array constitute a complete set of n-mers, (b) hybridizing the target polynucleotide to said overhangs of probe polynucleotides in one array to generate a target hybridization pattern, (c) hybridizing a reference polynucleotide to said overhangs of probe polynucleotides in a second array to generate a reference hybridization pattern, and (d) determining the presence of a mutation in the target polynucleotide by comparing the reference and target hybridization patterns. The term "complete set of n-mers" is defined in the specification at page 13, lines 3-5 as a set of single-stranded polynucleotides of "n" number of nucleotides wherein the set represents every possible combination of the "n" nucleotides.

The present method can be used to determine two or more unknown polynucleotide sequences to determine if they are identical without necessarily having to know the sequence of one of the polynucleotides to act as a reference. Unlike the traditional techniques which compare sequences, these methods compare hybridization patterns. Specification at page 15, lines 14-16. Thus, the invention does not require determination of the sequence of the target polynucleotide, nor does it necessitate that the sequence of the reference polynucleotide be known. Rather, the hybridization patterns of the reference and the target polynucleotides are directly compared. From this comparison one may determine the mutations in the target polynucleotide with a high degree of accuracy without sequencing the target polynucleotide. Specification at page 25, lines 23-29.

In contrast, the '134 patent is directed to methods for determining the nucleotide sequence of a nucleic acid by positional sequencing by hybridization. Col. 3, lines 25-62. In particular, the '134 patent attempted to solve problems with existing methods of nucleic acid sequencing. For example, col. 8, lines 1-12 describes an embodiment of the invention that is a method for determining a nucleotide sequence by hybridization. In other words, the '134 patent discloses a method of determining the specific base found at each position of a target polynucleotide; *i.e.*, sequencing it. The Examiner cites to column 12, lines 9-19 for the proposition that the '134 patent teaches the use of complete n-mer arrays. The '134 patent does suggest the use of n-mer arrays, but suggestion is limited to the use of those arrays for determining the nucleotide sequence of a nucleic acid molecule by positional sequencing hybridization and not to determining the presence of a mutation by comparing hybridization patterns.

The method of the present invention is different from that taught by the '134 patent. In its simplest form the present invention, an array containing a complete set of n-mers is hybridized with

a target polynucleotide to generate a target hybridization pattern, and an array containing a complete set of n-mers is hybridized with a reference polynucleotide to generate a reference hybridization pattern. These two hybridization patterns are then compared. Thus, two double-stranded polynucleotide patterns are compared. On the other hand, the '134 patent describes hybridizing a target nucleotide to an array wherein the sequence of each probe on the array is already known, in order to sequence the target nucleotide. Thus, in the '134 patent, a single strand of nucleic acid is compared to another single strand of nucleic acid on a single array.

The '134 patent also discloses a method of preparing customized probes that are used to prepare an array. Col. 3, line 63 - Col. 4, line 8; Fig. 10; Col. 9, line 64 through Col. 10, line 67. For example, customized probes are discussed at column 10, lines 23-67 as useful for identifying mutations. Furthermore, Example 12 (columns 17-18) discusses custom arrays of probes and their use in detecting mutations. Applicant respectfully asserts, however, that the customized probes discussed in the '134 patent as useful for determining the presence of a mutation do not in any way suggest the use of a probe array wherein each array constitutes a complete set of n-mers to determine the presence of a mutation. Furthermore, the Examiner conceded that the '134 patent does not teach hybridizing a reference polynucleotide to a second array and determining the presence of a mutation by comparing the reference and target hybridization patterns. (page 3, lines 6-8 of the September 1, 2000 Office Action)

Yershov *et al.* does not remedy the deficiencies of the '134 patent in that neither the '134 patent nor Yershov *et al.* teach or discuss the use of a probe array wherein each array constitute a complete set of n-mers to analyze mutations by comparing hybridization patterns. The Examiner, citing Yershov *et al.*, alleges that the comparison of reference and target hybridization patterns to determine the presence of a mutation was known and routinely practiced in the art at the time the claimed invention was made. Specifically, the Examiner alleges that Figure 3 of Yershov *et al.* teaches a method for determining the presence of a mutation in a target polynucleotide comprising hybridizing a target polynucleotide to one array and a reference polynucleotide to a second array and determining the presence of a mutation by comparing reference and target hybridization patterns.

The information presented in Figure 3 of Yershov *et al.* relates to the examination of DNA from blood from β-thalassemia patients. Lines 2-5 of the legend for Figure 3 illustrates the results from experiments using microchips with "six 100 X 100-μm elements . . . or four 40 X 40-μm

elements . . . of gel immobilized 10-mers. The 10-mers are indicated on the microchips by the number 1-6 and correspond to different β-thalassemia genotypes." The exact sequences of the six custom probes are presented in Figure 3. Thus, this experiment is limited to the use of custom arrays of at most six custom probes. Yershov *et al.* expands the potential use of custom arrays, but does not does not teach or discuss the use of a probe array wherein each array constitutes a complete set of n-mers to analyze mutations. There is no suggestion or motivation in the references to modify Yershov *et al.* to use a complete set of n-mers to determine the presence of a mutation in a target polynucleotide by comparing a reference and target hybridization pattern.

Therefore, it is respectfully submitted that claim 1, which recites providing at least two identical polynucleotide probe arrays, where each array constitutes a complete sets of n-mers, to determine the presence of a mutation in a target polynucleotide by comparing a reference and target hybridization pattern, is not *prima facie* obvious over the '134 patent either alone or in view of Yershov *et al.* Applicant's Representatives respectfully request that the Examiner withdraw this 35 U.S.C. § 103 rejection of claim 1. Claims 2-11 are dependent upon claim 1, and Applicant respectfully requests that the rejection of these claims be withdrawn for reasons stated above.

Claim 12

Claim 12 is directed to a method of determining whether two or more target polynucleotides are identical, comprising the steps of (a) providing at least two identical polynucleotide probe arrays, wherein each probe comprises a double stranded region and a single-stranded n-mer overhang region such that the overhangs in each array constitute a complete set of n-mers, (b) hybridizing the first target polynucleotide to said overhangs of probe polynucleotides in one array to generate a first hybridization pattern, (c) hybridizing a second target polynucleotide to said overhangs of probe polynucleotides in a second array to generate a second hybridization pattern, and (d) comparing the first and second hybridization patterns.

As discussed above, the '134 patent describes two inventions. The first is a method of positional sequencing, and the second is a method of using custom arrays. The '134 patent discusses the use of n-mer arrays for determining the nucleotide sequence of a nucleic acid molecule by positional sequencing hybridization. The '134 patent does not and not to determining whether two or more target polynucleotides are identical. Furthermore, the Examiner conceded that the '134

patent does not teach the method comprising two identical arrays wherein the target polynucleotide is hybridized to one array and a second target polynucleotide is hybridized to a second array. *See* page 5, lines 6-8 of the September 1, 2000 Office Action.

The Examiner, citing Yershov *et al.*, alleges that the comparison of hybridization patterns to determine if two or more polynucleotides are identical was known and routinely practiced in the art at the time the claimed invention was made. However, as previously described, this experiment illustrated in Figure 3 of Yershov *et al.* is limited to the use of custom arrays of at most six custom probes. Therefore, Yershov *et al.* does not does not teach or discuss the use of a probe array wherein each probe comprises a double stranded region and a single-stranded n-mer overhang region such that the overhangs in each array constitute a complete set of n-mers to analyze mutations.

Thus, Yershov *et al.* does not remedy the deficiencies of the '134 patent. Neither the '134 patent nor Yershov *et al.* teach or discuss the use of a probe array wherein each array constitutes a complete set of n-mers to determine if two or more polynucleotides are identical by comparing their hybridization patterns.

Therefore, it is respectfully submitted that claim 12, which recites comparing at least two target hybridization patterns on at least two identical polynucleotide probe arrays, where each array constitutes a complete sets of n-mers, is not *prima facie* obvious over the '134 patent either alone or in view of Yershov *et al.* Applicant's Representatives respectfully request that the Examiner withdraw this 35 U.S.C. § 103 rejection of claim 12. Claims 13-18 are dependent upon claim 12, and Applicant respectfully requests that the rejection of these claims be withdrawn for reasons stated above.

Conclusion

Applicant believes the claims are in condition for allowance and requests reconsideration of the application and allowance of the claims. The Examiner is invited to telephone the below-signed attorney at (612) 373-6961 to discuss any questions which may remain with respect to the present application.

Respectfully submitted,

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Commissioner of Patents, Washington, D.C. 20231, on this 28 day of February, 2001.

Candis B. Buending
Name

_____
Signature